

**INSTRUCTION MANUAL FOR
CHIRALPAK® AD-RH, AS-RH, AY-RH, AZ-RH
CHIRALCEL® OD-RH, OJ-RH, OX-RH, and OZ-RH**

<Reversed-Phase>

Please read this instruction sheet completely before using these columns

Column Description

<p align="center">AMYLOSE-BASED</p> <p align="center">Coated on 5 µm silica gel</p>		<p align="center">CELLULOSE-BASED</p> <p align="center">Coated on 5 µm silica gel</p>	
<p align="center">CHIRALPAK® AD-RH</p> <p align="center">Amylose tris(3,5-dimethylphenylcarbamate)</p>	<p align="center">CHIRALPAK® AS-RH</p> <p align="center">Amylose tris[(S)-α-methylbenzylcarbamate]</p>	<p align="center">CHIRALCEL® OD-RH</p> <p align="center">Cellulose tris(3,5-dimethylphenylcarbamate)</p>	<p align="center">CHIRALCEL® OJ-RH</p> <p align="center">Cellulose tris(4-methylbenzoate)</p>
<p align="center">CHIRALPAK® AY-RH</p> <p align="center">Amylose tris(5-chloro-2-methylphenylcarbamate)</p>	<p align="center">CHIRALPAK® AZ-RH</p> <p align="center">Amylose tris(3-chloro-4-methylphenylcarbamate)</p>	<p align="center">CHIRALCEL® OX-RH</p> <p align="center">Cellulose tris(4-chloro-3-methylphenylcarbamate)</p>	<p align="center">CHIRALCEL® OZ-RH</p> <p align="center">Cellulose tris(3-chloro-4-methylphenylcarbamate)</p>

Shipping Solvent: **Water/ACN = 60/40 (v/v)**

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, were included with the column when purchased.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC system, including the injector and the injection loop, must be flushed with a solvent compatible with the column and its storage solvent prior to connecting the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.

If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	150 x 4.6 mm i.d. Analytical Columns	250 x 21 mm i.d.① Semi-Prep Columns
Guard	//	10 x 4.0 mm i.d. Guard Cartridge	//
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate②	0.1-0.5 ml/min	0.5-2.5 ml/min	21 ml/min (21 mm i.d.)
Pressure Limitation③	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 500 ml (for 250 x 21 mm i.d) of eluent at the beginning of each preparative work.

② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

③ The column pressure is the total pressure minus the system pressure. At a given temperature, the column back pressure is linearly proportional to the flow rate.

Operating Procedure

 **Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.**

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-RH.

	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALPAK® AZ-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH	CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH
	ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM NH ₄ HCO ₃ aq. pH 9.0 adjusted with a basic additive ❶
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions ACN		60% 40% ❹

NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases (Section B)

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALPAK® AZ-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH	CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH
	ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H ₃ PO ₄ aq. pH 2.0 OR 100mM KPF ₆ (or NaPF ₆) aq. pH 2.0 adjusted with H ₃ PO ₄	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❸ OR 100mM KPF ₆ (or NaPF ₆) aq.
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions ACN		60% 40% ❹

NOTE 2: The concentration of all the buffering salt should be less than 500mM.

- ❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.
- ❷
 - ❑ It is recommended to use ACN to start the investigation
 - ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 65-70% EtOH ≈ 75-80% MeOH.
 - ❑ The use of other organic solvents has not been investigated and could be harmful to the columns.
 - ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.
- ❸
 - ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
 - ❑ Lowering the column temperature may increase the retention time and the selectivity.

- ❑ Increasing the column temperature and decreasing the flow rate may increase the resolution.
- ④ ❑ To maximize column life, the use of a guard cartridge is essential when basic conditions are employed.
 - ❑ The use of strongly basic conditions ($> \text{pH } 9$) must be avoided, as they are known to damage the silica gel matrix.
 - ❑ When these columns are used at $\text{pH} > 7$, **the temperature should be maintained between 5°C and 25°C for maximum column life.**
- ⑤ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

- ⑥ Do not use a phosphate buffer for $\text{pH} > 8$. When $\text{pH } 9$ is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

- Preparation of pH 2 Phosphate buffer:

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.
- Preparation of pH 2 KPF_6 (NaPF_6) solution:

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g NaPF_6 / FW 167.95, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.
- Preparation of pH 9 Ammonium bicarbonate solution:

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml with HPLC grade water

Solution B: Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
** DEA tends to give better peak shape than other bases.*

Adjust the pH of solution A to a value of 9.0 using solution B.
- Preparation of pH 8 Phosphate buffer:

Solution A: 20mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.
- Preparation of pH 9 Borate buffer:

Solution A: 20mM sodium tetraborate decahydrate
3.81g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ Samples should preferably be dissolved in the mobile phase. The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.
- ❑ Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, e.g. Water/ACN 60:40 (v/v).
- ❑ If the column is contaminated with non-eluted components, wash it with a mobile phase that does not contain any salts / buffers, then with 100% ACN for 2 hours at 0.5ml/min. Alternatively, if the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.
- ❑ All salts must be flushed out from the HPLC system and column before changing to 100% ACN or 100% methanol.
- ❑ Use Water/ACN 60:40 (v/v) to store the column, at room temperature

Important Notice

⇒ STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED, because they are likely to damage the silica gel used in these columns.

Operating these columns in accordance with the guidelines outlined here will result in a long column life.

⇒ If you have any questions about the use of these columns, or encounter a problem, contact:

In the USA: questions@cti.daicel.com or call 800-6-CHIRAL

In the EU: cte@cte.daicel.com or call +33 (0) 3 88 79 52 00

In India: chiral@chiral.daicel.com or call +91 84 1866 0700 & 703

Locations:

North/Latin America

Chiral Technologies, Inc.
1475 Dunwoody Dr., Ste. 310
West Chester, PA 19380
800 6 CHIRAL
Tel: 610-594-2100
Fax: 610-594-2325
chiral@cti.daicel.com
www.chiraltech.com

Europe

Chiral Technologies Europe SAS
Parc d'Innovation
160, Bd Gonthier d'Andernach CS 80140
67404 Illkirch Cedex, France
Tel: +33 (0) 3 88 79 52 00
Fax: +33 (0) 3 88 66 71 66
cte@cte.daicel.com
www.chiraltech.com

India

Daicel Chiral Technologies (India) Pvt Ltd.
Survey No.542/2, IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

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